

(19)



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 0 855 444 A2

(12)

EUROPEAN PATENT APPLICATION

(43) Date of publication:
29.07.1998 Bulletin 1998/31

(51) Int Cl.⁶: C12N 15/57, C12N 9/64,
C12N 1/21, C12N 5/10,
C07K 16/40, A61K 48/00,
A61K 39/395, A61K 31/70,
A61K 38/48, C12Q 1/68,
C12Q 1/37

(21) Application number: 98300573.7

(22) Date of filing: 27.01.1998

(84) Designated Contracting States:
AT BE CH DE DK ES FI FR GB GR IE IT LI LU MC
NL PT SE

(30) Priority: 28.01.1997 GB 9701684

(71) Applicants:

- SMITHKLINE BEECHAM PLC
Brentford, Middlesex TW8 9EP (GB)
- SMITHKLINE BEECHAM CORPORATION
Philadelphia Pennsylvania 19103 (US)

(72) Inventors:

- Powell, David J.,
c/o SmithKline Beecham Pharm.
King of Prussia, PA 19460 (US)

- Smith, Trudi S., c/o SmithKline Beecham Pharm.
King of Prussia, PA 19460 (US)
- Chapman, Conrad G.,
c/o SmithKline Beecham Pharm.
Harlow, Essex CM19 5AW (GB)
- Murphy, Kay SmithKline Beecham Pharm.
Harlow Essex CM19 5AW (GB)

(74) Representative: Crump, Julian Richard John et al
fJ Cleveland,
40-43 Chancery Lane
London WC2A 1JQ (GB)

(54) Aspartic proteinase 2 (ASP2)

(57) ASP2 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing ASP2 polypeptides and polynucle-

otides in the design of protocols for the treatment of Alzheimer's Disease, cancer, and prohormone processing, among others, and diagnostic assays for such conditions.

Description

This application claims the benefit of U.K. Application No. 9701684.4, filed January 28, 1997, which is herein incorporated by reference in its entirety.

5

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to Aspartic Proteinase family, hereinafter referred to as ASP2. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

10

BACKGROUND OF THE INVENTION

There are currently five known human aspartic proteases, namely, pepsin, gastricsin, cathepsin D, cathepsin E and renin, and these have widely varying functions. Pepsin and gastricsin are involved in nutritive processes in the stomach, cathepsin D is involved in protein turnover in many cell types, and renin has the highly specific function of angiotensin production from its precursor form, angiotensinogen. The precise role of cathepsin E remains to be confirmed, although its location in some epithelial cells types has indicated a role in antigen processing. It may also be involved in certain inflammatory conditions, such as *Helicobacter pylori* infection in the stomach. This indicates that the Aspartic Proteinase family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of Aspartic Proteinase family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, Alzheimer's Disease, cancer, and prohormone processing.

25

SUMMARY OF THE INVENTION

In one aspect, the invention relates to ASP2 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such ASP2 polypeptides and polynucleotides. Such uses include the treatment of Alzheimer's Disease, cancer, and prohormone processing, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with ASP2 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate ASP2 activity or levels.

35

DESCRIPTION OF THE INVENTION**Definitions**

The following definitions are provided to facilitate understanding of certain terms used frequently herein. "ASP2" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO: 2 or an allelic variant thereof.

"ASP2 activity or ASP2 polypeptide activity" or "biological activity of the ASP2 or ASP2 polypeptide" refers to the metabolic or physiologic function of said ASP2 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said ASP2.

"ASP2 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide"

refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity, as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heijne, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990). The BLAST X program is publicly available from NCBI

and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

- 5 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
 Comparison matrix: BLOSUM62 from Hentikoff and Hentikoff, *Proc. Natl. Acad. Sci. USA.* 89:10915-10919 (1992)
 Gap Penalty: 12
 Gap Length Penalty: 4
- 10 A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polypeptide comparisons (along with no penalty for end gaps).

Preferred parameters for polynucleotide comparison include the following:

- 10 1) Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)
 Comparison matrix: matches = +10, mismatch = 0
 15 Gap Penalty: 50
 Gap Length Penalty: 3

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for polynucleotide comparisons.

Preferred polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polynucleotide reference sequence of SEQ ID NO:1, wherein said reference sequence may be identical to the sequence of SEQ ID NO: 1 or may include up to a certain integer number of nucleotide alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO: 1 by the numerical percent of the respective percent identity and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

Preferred polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said reference sequence may be identical to the sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO :2 by the numerical percent of the respective percent identity and subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids in SEQ ID NO:2, and y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85 %, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

Polypeptides of the Invention

In one aspect, the present invention relates to ASP2 polypeptides (or ASP2 proteins). The ASP2 polypeptides include the polypeptide of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO:2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Also included within ASP2 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably ASP2 polypeptide exhibit at least one biological activity of ASP2.

The ASP2 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the ASP2 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned ASP2 polypeptides. As with ASP2 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of ASP2 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of ASP2 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate ASP2 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the ASP2, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The ASP2 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to ASP2 polynucleotides. ASP2 polynucleotides include isolated polynucleotides which encode the ASP2 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, ASP2 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO:1 encoding a ASP2 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS: 1 and 3. ASP2 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under ASP2 polynucle-

otides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides poly-nucleotides which are complementary to such ASP2 polynucleotides.

ASP2 of the invention is structurally related to other proteins of the Aspartic Proteininase family, as shown by the results of sequencing the cDNA encoding human ASP2. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 1 to 1503) encoding a polypeptide of 501 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 48.7% identity (using FASTA (GCG)) in 460 amino acid residues with ASP1, Novel Aspartic Proteininase, (U.S. Serial No. Unassigned, Attorney Docket Number GH70262, filed October 6, 1997). The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 59.2% identity (using FASTA (GCG)) in 1516 nucleotide residues with ASP1 Novel Aspartic Proteininase (U.S. Serial No. Unassigned, Attorney Docket number GH70262, filed October 6, 1997). Thus, ASP2 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1^a

ATGG CCCAAG CCCTG CCGTGG CT CCT GCT GT GGAT GGG CG CGGGAGT GCT GCTG CCCAOGG CACCCAG
CACGG CAT CCGG CT GCCCCCTG CG CAG OG GCT GGGGGG CG CCCCCCTGGGG CT GCGG CTG CCCCGGGAG
ACCGAGAAGAG CCGGAGGAG CCGG CGGGAGGGG CAG CTT GT GGAGAT GGT GGACAACCT GAGGGC
AAGT CGGGG CAGGG CT ACT ACCT GGAGAT GACCGT GGG CAG CCCCCCG CAGACG CT CAACAT CCTGGTG
GAT ACAGG CAG CAGT AAC TTTG CAGT GGGT GCT GCCCCCCCACCCCT CCTG CAT CG CT ACT ACCAGAGG
CAG CT GT CCAG CA CAT ACGGGACCT CGGAAGGGT GT GT AT GAG CCCT ACACCAGGG CAAGT GGGAA
GGGGAG CT GGG CA COGACCT GGT AAG CAT CCCCCAT GG CCCCACCGT CACT GT GCGT GCAA ACATT GCT
GCCAT CACT GAAT CAGACAAGTT CTT CAT CAACGG CT CCAACT GGGAAGG CAT CCTGGGG CT GG CCT AT
GCT GAG ATT G CCAGG CCTGACGACT CCCC GGAG CCTT CTT GACT CT CTGGT AAAG CAGACCCACGTT
CCCCAACCT CTT CT CCCTG CAG CTT GT GGGT GCT GGGT CCCCCC CAACCGAT CTGAAGTG CT GG CCT CT
GT CGGAGGGAG CAT GAT CATT GGAGGT AT CGACCACT CG CT GT ACACAGG CAGT CT CTGGT AT ACACCC
AT CGGG CGGGAGT GGT ATT AT GAGGT GAT CATT GT G CGGGT GGAGAT CAAT GGACAGGAT CT GAAAATG
GACT GCAAGGAGT ACAACT AT GACAAGAG CATT GT GGACAGT GG CACCACCAACCTT CGTTG CCCAAG
AAAGT GTTTGAAG CT G CAGT CAAAT CCAT CAAGG CAG CCT CCT CCACGGAGAAGTT CCTGATGGTT C
TGG CT AGGAGAG CAG CT GGT GT GCT GG CAAG CAGG CACCACCCCTT GGAACATTT CCCAGT CAT CT CA
CT CT ACCT AT GGGT GAGGTT ACCAACCAGT CCTT CCG CAT CACCAT CCTT CCG CAG CAAT ACCT GGG
CCAGT GGAAGAT GT GG CCACGT CCCAAGACGACT GTT ACAAGTTGCCAT CT CACAGT CAT CCA CGGGC
ACT GTT AT GGGAG CT GTT AT CAT GGAGGG CTT CT ACCT GT CTT GAT CGGG CCCG AAAACGAATT GGC
TTT GCT GT CAG CG CTT G CCAT GT G CACGAT GAGTT CAGGACGG CAG OG GT GGAAGG CCCC TT GT CACC
TT GGACAT GGAAGAGCT GT GG CT ACAACATT CCACAGACAGAT GAGT CAACCT CAT GACCAT AG CCT AT
GT CAT GG CTG CCAT CT G CG CCCT CTT CAT GCT G CCACT CTG CCT CAT GGT GT GT CAGT GG CG CT G CCT C
CG CT G CCGT G CG CCAG CAG CAT GAT GACIT TG CTG AT GACAT CT CCCC CTG CT GAAGTGAGGAGG CCCATGG
GAGAAAGAT AGAGATT CCCCTGGGACCAACCT CGTGGTT CACTTGGT CACAAGTAGGAGA CACAGA
TGG CACCT GT GG CCAGAGCACCT CAGGACCC CCCCACCCACAAATG CCT CT G CCGT GAT GGAGAAGG
AAAAGG CT GG CAAGGT GGGTT CCAGGGACT GT ACCT GT AGGAAACAGAAAAGAGAAGAAAGAAG CACT C
TG CT GG CGGGAAAT ACT CTT GGT CACCT CAAATT TAAGT CGGGAAATT CTG CTG CTT GAAACTT CAG CCC
TGAACCTTGT CCACCAT T CTTTAATT CT CCAACCCAAAGT ATT CTT CTTT CTT AGTTT CAGAAGT
ACT GG CAT CACACG CAGGTT ACCT TG CGT GT CCCT GT GGT ACCCGGG CAGAGAAGAGACCAAG CTT
GTTT CCCC CTG CT GG CCAAAGT CAGT AGGAGAGGAT G CACAGTTG CT ATTT G CTT AGAGACAGGGACT

5 GT AT AAA CAAG CCT AAC ATT GGT GCAAAGATT CCT CTT GAATT AAAAAAAA ACT AG ATT GACT ATT
T AT ACAAA TGGGG CGG CTGGAAAGAGGAGAAGGAGAGGAGT ACAAAAGACAGGAAT AGT GGGAT CAA
AG CT AGGAAAGG CAGAACACAACCACT CACCACT CCT AGTTT AGACCT CAT CT CCAAGAT AG CAT CC
CAT CT CAGAAGAT GGGT GTT GTTT CAAT GTTT CT TTT CTGTGGTTGCAG CCTGACCAAAAGT GAGAT
GGGAAGGG CTT AT CT AG CCAAAGAG CT CTTTTAG CT CT CTT AAATGAAGT GCCC ACT AAGGAAGTT C
CACT TGAAACACAT GGAATT CT GCCAT ATT AATT CCATT GT CT CT AT CT GGAACCACCC TT AAT CT C
TACAT AT GATT AGGT CCAG CACTT GAAAAT ATT CCT AACCNNAATT GNCCTGGGG CTT GCNGN
CCAGGT GCT AAAAGGG NTTGGGT AGG NGNCCNCT NT AT NT NAT NCCT NAAAAGGT TANNG

10 15 20 25 30 ^a A nucleotide sequence of a human ASP2 (SEQ ID NO: 1).

Table 2^b

MAQALPWLLLWMGAGVLP AHGTQHGI RLPL RSGLGGAP LGL RLP RETDEEPEEPG RRG SFVEMVD NL RG
K SGQGYYVEMTVG SPPQT LN ILVDTG SSNF AVGA AP HP FLH RY Q RQL S ST Y RDL RKG VYE PY T QGKWE
GELGTDLV S IHPGP NVT V RANI AAI TE SDKFFI NG SNWEGIL GLAYAE I A PDD SLEP FFD SLVKQTHV
P NLF SLQL CGAGFPL NQ SEVLA SVGG SMI IGGIDH SLYTG SLWYTP I RREWYYEVII IV RVE I NGQD LKM
D CKEY NYDK SIVD SGTT NL RLPKKVFEAAVK SIKAA SSTEKFPDGFWLGEQLVCWQAGTT PWNI FPVI S
LYLMGEVT NQ SF RIT I LPQQYL R PVED VAT SQDD CYKFAI SQ SST GTVMGAVI MEGFYVVFD RARK RIG
FAV SACHVHDEF RT AAVEGP FVT LD MED CGY NI PQT DE ST LMT I AYVMAAI CALFMLPL CLMV CQW RCL
RCL RQHDD FADD I SLLK

^b An amino acid sequence of a human ASP2 (SEQ ID NO: 2).

35 One polynucleotide of the present invention encoding ASP2 may be obtained using standard cloning and screening,
from a cDNA library derived from mRNA in cells of human pancreas and brain, using the expressed sequence tag
(EST) analysis (Adams, M.D., et al. *Science* (1991) 252:1651-1656; Adams, M.D. et al., *Nature*, (1992) 355:632-634;
Adams, M.D., et al., *Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural
sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

40 The nucleotide sequence encoding ASP2 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding
sequence contained in Table 1 (nucleotide number 1 to 1503 of SEQ ID NO:1), or it may be a sequence, which as a
result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

45 When the polynucleotides of the invention are used for the recombinant production of ASP2 polypeptide, the poly-
nucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding
sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding
a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For
example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred
embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE
vector (Qiagen, Inc.) and described in Gentz et al., *Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The
polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences,
splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

50 Further preferred embodiments are polynucleotides encoding ASP2 variants comprising the amino acid sequence
of ASP2 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are
substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is
contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

55

Table 3^f

5	GG CAG CTT GT GGAGAT GGT GGACAACCT GAGGGG CAAGT CGGGG CAGGG CT ACT ACGT GGAGAT GACC GTGGG CAG CCCCCCG CAG ACG CT CAACAT CCTGGT GGAT ACAGG CAG CAGT AACT TTG CAGT GGGT GCT GCC CCCCACCCCTT CCTG CAT CG CT ACT ACCAGAGG CAG CTGT CCAGCACATACCGGGACCT CCGGAAG
10	GGT GTGT ATGAG CCCT ACACCCAGGG CAAGT GGGAGGGAG CTGGG CACCGACCT GGT AAGCAT CCCC CATGG CCCCCAACGT CACT GTG CGTG CCAA CATT GCTGCCAT CACTGAAT CAGACAAGTT CTT CAT CAAC GGCT CCAACTGGGAAGG CAT CCTGGGG CTGG CCTATG CTGAGATTGCCAGG CCTGACGACT CCCGGAG
15	CCTT CTTT GACT CT CTGGT AAAG CAGACCCACGTT CCCAACCT CTT CT CCCTG CAG CTT GT GGT GCT GGCTT CCCCCT CAACCAGT CTGAAGT GCTGG CCTCTGT CGGAGGGAG CATGAT CATTGGAGGTAT CGAC CACT CG CTGT ACACAGG CAGT CT CTGGT ATACACCCAT CGGG CGGGAGT GGT ATT ATGAGGTGAT CATT
20	GTG CGGGT GGAGAT CAATGGACAGGAT CTGAAATGGACTG CAAGGAGT ACAACT ATGACAAGAG CATT GTGGACAGTGG CACCACCAACCTT CGTTG CCCCAGAAAGT GTTTGAAGCTG CAGT CAAAT CCAT CAAG GCAG CCT CT CCA CGGGAGAAGTT CCCTGATGGTT CTGG CT AGGAGAG CAG CTGGT GTG CTGG CAAGCA
25	GGCACCA CCCCTT GGAACATTTC CCCAGT CAT CT CACT CT ACCTAATGGGTGAGGTTACCAACCAAGT CCT TT CCGCAT CACCAT CCTT CCGCAG CAATACCT GGGCCAGT GGAAGAT GTGGCCACGT CCCAAGACGAC TGTTACAAGTTGCCAT CT CACAGT CAT CCACGGG CACTGTT ATGGGAGCTGTT AT CATGGAGGG CTT C
30	TACGTT GT CTTGAT CGGG CCGAAAAGAATTGGCTTTG CTGT CAG CG CTTGCCAT GTG CACGATGAG TT CAGGA CGGCAG CGGT GGAAGG CCCCCTTGT CACCTGGACAT GGAAGACT GTGG CT ACAACATT CCA CAGACAGATGAGT CAACCCCT CATGACCAT AG CCT ATGT CATGG CTGCCAT CTG CG CCCT TTT CATG CTG
35	CCACT CTG CCT CATGGT GTGT CAGTGG CG CTG CCT CCG CTGCCGT CGCCAGACAATGGATGACTT GCT GATGACAT CT CCCTG CT GAAGT GAGGAGG CCCATGGGAGAAAGATAGAGATT CCCCTGGGACACACCT COGTGGTT CACTTGGT CACAAGT AGGAGACACAGATGGCACCT GTGGCCAGAGCACCT CAGGACCCCT C CCCACCCACCAAATG CCT CTG CCGT GATGGAGAAGGAAAGG CTGG CAAGGTGGTT CCAGGGACTGTA

40

45

50

5 CCTGTAGGAAACAGAAAAGAGAAGAAAGCACT CTGCTGGCGGGAATACT CTTGGTCACCTCAAATT
 TAAGT CGGGAAATT CTGCTGCTTGAAACTT CAGCCCTGAACCTTGT CCACCATT CTTAAATTCTCC
 AACCCAAAGTATTCTTCTTTCTTAGTTT CAGAAGTACTGGCATCACACG CAGGTTACCTTGGCGTGTG
 10 TCCCTGTGGTACCCGGG CAGAGAACAGAACAGCTTGTCCCTGCTGGCAAAGT CAGTAGGAGAGGA
 TG CACAGTTGCTATTGCTT AGAGACAGGGACTGTATAAACAAAGCCTAACATTGGTCAAAGATTGC
 CT CTTGAATTAAAAAAACTAGATTGACTATTTATACAAATGGGGGGCGCTGGAAAGAGGAGAAGG
 AGAGGGAGTACAAGACAGGAATAGTGGGATCAAAGCTAGGAAAGGCAGAAACACAACCACTCACCAG
 15 T CCTAGTTTAGACCTCATCTCCAAGATAGCATCCCATCTCAGAAGATGGGTGTTGTTTCAATGTTT
 CTTTCTGTGGTTGCAGCCTGACCAAAAGTGAGATGGGAAGGGCTTATCTAGCCAAAGAGCTCTTTTT
 AGCTCTCTAAATGAAGTGCCACTAAGGAAGTTCCACTGAAACACATGGAATTCTGCCATATTAAATT
 TCCATTGTCTCTATCTGGAACCACCTTAATCTCTACATATGATTAGGTCCAGCACTGAAAATATTCT
 20 CTAAACCNAATTGNCTGGGGCTTGCNGNCCAGGTGCTAAAGGGNTGGTAGGNGNCNCTNT
 ATNTNATNCNTNAAAAGGTTANNG

^c A partial nucleotide sequence of a human ASP2 (SEQ ID NO: 3).

25

Table 4^d

30 GSFVEMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHFLHRYYQRQLSSTYRDLRK
 GVYEPYTQGKWEDELGTDLVSPHGPNTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDELE
 PFFD SLVKQTHVPNLFSQLQGAGFPLNQSEVLA SVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVII
 VRVEINQDILKMDCKEINYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASPREKFPDGFWLGEQLVCWQA
 35 GTTPWNI FPVI SLYLMGEVTNQSF RITILPQQYL RPVEDVAT SQDDCYKFAISQSSTGTVMGAVIMEGF
 YVVFDRAKRRIGFAVSACHVHDEFRTAAVEGP FVTLD MEDCGYNIPQTDESTLMTIAYVMAAI CALFML
 PLCLMV CQWRCLRCLRQTMDDFADDISLLK. GGPWEKD RDSPGTTPPWFTLVT SRRHFWHLWPEHLRTL
 40 PTHQMPLP. WRKRLARWVPGTVPVGNRKEKKEALCWREYSWSPQI. VGKFCCCLKLQP. TFVHH SFKFS
 NPKYSSFLSF RSTGIT RRLPW RVSLWYPG REETKLV SLLAKV SRRGCTVCYLL. RQGLYKQA. HWCKDC
 LLN. KKKLD. LFIQMGAAGK RRRRG STKTGNNSGIARKGRNTTHQS. F. TSSPRHPIDESDGCCFQCF
 45 LFCGCSLTKESEMGRAYLAKELFFSSLK. SAH. GSST. THGI SAILISIVSIWNHPLISTYD. VQHLKIF
 LTXIXLGGFAXQVLKGXG. XXXXXXXXLKRLX

^d A partial amino acid sequence of a human ASP2 (SEQ ID NO: 4).

50 The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

55 Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof (including that of SEQ ID NO:3), may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding ASP2 polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the ASP2 gene. Such hybridization techniques are known to those of skill in

the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

- 5 In one embodiment, to obtain a polynucleotide encoding ASP2 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, ASP2 polynucleotides of the present invention further 10 include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO:3). Also included with ASP2 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 15 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

20 **Vectors, Host Cells, Expression**

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such 25 proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL*, 2nd Ed., Cold Spring 30 Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli*, *Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila S2* and *Spodoptera Sf9* cells; animal cells such as CHO, COS, HeLa, C127,3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from 40 yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well 45 as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL* (*supra*).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the ASP2 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If ASP2 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered.

ASP2 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide

is denatured during isolation and or purification.

Diagnostic Assays

5 This invention also relates to the use of ASP2 polynucleotides for use as diagnostic reagents. Detection of a mutated form of ASP2 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of ASP2. Individuals carrying mutations in the ASP2 gene may be detected at the DNA level by a variety of techniques.

10 Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled ASP2 nucleotide sequences. Perfectly

15 matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al.*, *Science* (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al.*, *Proc Natl Acad Sci USA* (1985) 85: 4397-4401.

20 In another embodiment, an array of oligonucleotides probes comprising ASP2 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al.*, *Science*, Vol 274, pp 610-613 (1996)).

25 The diagnostic assays offer a process for diagnosing or determining a susceptibility to Alzheimer's Disease, cancer, and prohormone processing through detection of mutation in the ASP2 gene by the methods described.

In addition, Alzheimer's Disease, cancer, and prohormone processing, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of ASP2 polypeptide or ASP2 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well

30 known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an ASP2 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

35 Thus in another aspect, the present invention relates to a diagnostic kit for a disease or susceptibility to a disease, particularly Alzheimer's Disease, cancer, and prohormone processing, which comprises:

- (a) a ASP2 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a ASP2 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
- 40 (d) an antibody to a ASP2 polypeptide, preferably to the polypeptide of SEQ ID NO: 2. It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

45 The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, *Mendelian Inheritance in Man* (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

55 A chromosomal loci of 11q22 has been inferred for ASP2 by homology (99% in 210 nucleotides) with Genbank Locus G24698 (Human STS WI-14206).

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the ASP2 polypeptides. The term "immunospecific" means that the antibodies have substantially greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

5 Antibodies generated against the ASP2 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. 10 Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al.*, *Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.*, *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, pp. 77-96, Alan R. Liss, Inc., 1985).

15 Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

16 The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

17 Antibodies against ASP2 polypeptides may also be employed to treat Alzheimer's Disease, cancer, and prohormone processing, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with ASP2 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from Alzheimer's Disease, cancer, and prohormone processing, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering ASP2 polypeptide via a vector directing expression of ASP2 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a ASP2 polypeptide wherein the composition comprises a ASP2 polypeptide or ASP2 gene. The vaccine formulation may further comprise a suitable carrier. Since ASP2 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain antioxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

45 The ASP2 polypeptide of the present invention may be employed in a screening process for compounds which activate (agonists) or inhibit activation of (antagonists, or otherwise called inhibitors) the ASP2 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess identify agonist or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural or modified substrates, ligands, enzymes, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al.*, *Current Protocols in Immunology* 1(2):Chapter 5 (1991).

50 ASP2 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate ASP2 polypeptide on the one hand and which can inhibit the function of ASP2 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and prohormone processing. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as Alzheimer's Disease, cancer, and prohormone processing.

55 In general, such screening procedures may involve using appropriate cells which express the ASP2 polypeptide

or respond to ASP2 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the ASP2 polypeptide (or cell membrane containing the expressed polypeptide) or respond to ASP2 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for ASP2 activity. In addition, all aspartic proteinases are inhibited by pepstatin. Therefore, pepstatin inhibitory assays may also be employed with the present invention as a method of detection or as a screening assay.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the ASP2 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the ASP2 polypeptide, using detection systems appropriate to the cells bearing the ASP2 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing a ASP2 polypeptide to form a mixture, measuring ASP2 activity in the mixture, and comparing the ASP2 activity of the mixture to a standard.

The ASP2 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of ASP2 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of ASP2 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of ASP2 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The ASP2 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the ASP2 is labeled with a radioactive isotope (eg 125I), chemically modified (eg biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of ASP2 which compete with the binding of ASP2 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential ASP2 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, enzymes, receptors, etc., as the case may be, of the ASP2 polypeptide, e.g., a fragment of the ligands, substrates, enzymes, receptors, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for ASP2 polypeptides; or compounds which decrease or enhance the production of ASP2 polypeptides, which comprises:

- (a) a ASP2 polypeptide, preferably that of SEQ ID NO:2;
- (b) a recombinant cell expressing a ASP2 polypeptide, preferably that of SEQ ID NO:2;
- (c) a cell membrane expressing a ASP2 polypeptide; preferably that of SEQ ID NO: 2; or
- (d) antibody to a ASP2 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

45 Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, Alzheimer's Disease, cancer, and pro-hormone processing, related to both an excess of and insufficient amounts of ASP2 polypeptide activity.

If the activity of ASP2 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the ASP2 polypeptide, such as, for example, by blocking the binding of ligands, substrates, enzymes, receptors, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of ASP2 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous ASP2 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the ASP2 polypeptide.

In another approach, soluble forms of ASP2 polypeptides still capable of binding the ligand in competition with endogenous ASP2 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the ASP2 polypeptide.

In still another approach, expression of the gene encoding endogenous ASP2 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al.*, *Nucleic Acids Res* (1979) 6:3073; Cooney *et al.*, *Science* (1988) 241:456; Dervan *et al.*, *Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of ASP2 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates ASP2 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of ASP2 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of ASP2 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of ASP2 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo*, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Examples

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples illustrate, but do not limit the invention.

Cloning:

Rapid amplification of cDNA ends polymerase chain reaction technology (RACE PCR) was used to identify the

missing 5' cDNA sequence of the aspartyl protease 2 gene. The source of cDNA template for the amplification reactions was a range of Marathon-Ready™ cDNA preparations (Clontech Laboratories, Inc., 1020 East Meadow Circle, Palo Alto, CA 94303-4230, USA.). These Marathon-Ready cDNAs are essentially cDNA libraries which have oligonucleotide adaptors ligated onto them. This allows the researcher to perform 5' RACE PCR using two primers, one complementary to a region of known sequence in the gene of interest and the other complementary to the ligated adaptor; resulting in an extension to the known sequence at the 5'end. PCR was performed using AmpliTaq® Gold DNA polymerase (Perkin-Elmer Corp).

It was found to be necessary to include 5% Dimethylsulphoxide in the reaction buffer for successful amplification, probably due to the high GC nucleotide content of this region of DNA.

The DNA sequence was cloned and a region of DNA was confirmed (nucleotides 1-273 in Table 1) at the 5' end of the Asp2 gene as extending from the start codon to overlap with the previously identified EST sequences. This novel sequence was identified in cDNA templates from seven human tissues, heart, leukocyte, mammary gland, spleen, skeletal muscle, thymus and aorta.

15 Northern Analysis:

A human Multiple Tissue Northern blot (MTN catalogue number 7760-1) (Clontech) was hybridized with an Asp-2 specific probe (of 325 nucleotides in length) generated by PCR, using the specific oligonucleotides 5' GATGAGTCAGGACGGCAG 3' (SEQ ID NO:5) and 5' GGTGCCATATGTGTCTCC 3' (SEQ ID NO:6). The probe was radiolabelled by incorporation of ^{32}P -dCTP during PCR amplification, and the labelled PCR product was subsequently purified using the Qiagen PCR Purification Kit. After a 1 hour prehybridization, hybridization was carried out for 2 hours using ExpressHyb buffer (Clontech) at 68°C, and the labelled probe was added to a final concentration of 1×10^6 cpm/ml. After hybridization, the membrane was washed twice in 2×SSC/ 0.05% SDS for 20 minutes, and twice in 0.1×SSC/ 0.1% SDS at 50°C for 20 minutes. The membrane was then wrapped in plastic wrap and exposed to X-ray film at -70°C with two intensifying screens. This revealed that the highest expression (tissues examined were heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas) of Asp2 was in the pancreas, followed by the brain.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

30

35

40

45

50

55

SEQUENCE LISTING

5

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham p.l.c. and
SmithKline Beecham Corporation

10

(ii) TITLE OF THE INVENTION: ASP2

15

(iii) NUMBER OF SEQUENCES: 6

(iv) CORRESPONDENCE ADDRESS:

- (A) ADDRESSEE: F J Cleveland & Company
(B) STREET: 40/43 Chancery Lane
(C) CITY: London
(D) COUNTY:
25 (E) COUNTRY: United Kingdom
(F) POST CODE: WC2A 1JQ

30

(v) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
35 (D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:

- 40 (A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 20-JAN-1998
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:

- 45 (A) APPLICATION NUMBER: UK 9701684.4
(B) FILING DATE: 28-JAN-1997

50

(viii) ATTORNEY/AGENT INFORMATION:

- 55 (A) NAME: CRUMP, Julian Richard John
(B) GENERAL AUTHORISATION NUMBER: 37129
(C) REFERENCE/DOCKET NUMBER: GH-70368

(ix) TELECOMMUNICATION INFORMATION:

- 5
 (A) TELEPHONE: +44 171 405 5875
 (B) TELEFAX: +44 171 831 0749
 (C) TELEX:

10 (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- 15
 (A) LENGTH: 2541 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

25	ATGGCCCAAG CCCTGCCCTG GCTCCTGCTG TGGATGGCG CGGGAGTGCT GCCTGCCAC	60
	GGCACCCAGC ACGGCATCCG GCTGCCCTG CGCAGCGGCC TGGGGGGCGC CCCCCCTGGGG	120
	CTGCGGCTGC CCCGGGAGAC CGACGAAGAG CCCGAGGAGC CCGGCCGAG GGGCAGCTTT	180
30	GTGGAGATGG TGGACAACCT GAGGGGCAAG TCAGGGCAGG GCTACTACGT GGAGATGACC	240
	GTGGGCAGCC CCCCGCAGAC GCTAACATC CTGGTGGATA CAGGCAGCAG TAACCTTGCA	300
	GTGGGTGCTG CCCCCCACCC CTTCCTGCAT CGCTACTACC AGAGGCAGCT GTCCAGCACA	360
	TACCGGGACC TCCGGAAGGG TGTGTATGAG CCCTACACCC AGGGCAAGTG GGAAGGGAG	420
35	CTGGGCACCG ACCTGGTAAG CATCCCCAT GGCCCCAACG TCACTGTGCG TGCCAACATT	480
	GCTGCCATCA CTGAATCAGA CAAGTTCTTC ATCAACGGCT CCAAATGGGA AGGCATCCTG	540
	GGGCTGGCCT ATGCTGAGAT TGCCAGGCCT GACCGACTCCC TGGAGCCTTT CTTTGACTCT	600
40	CTGGTAAAGC AGACCCACGT TCCCAACCTC TTCTCCCTGC AGCTTTGTGG TGCTGGCTTC	660
	CCCCTCAACC AGTCTGAAGT GCTGGCCTCT GTGGAGGGGA GCATGATCAT TGGAGGTATC	720
	GACCACTCGC TGTACACAGG CAGTCTCTGG TATACACCCA TCCGGCGGGA GTGGTATTAT	780
	GAGGTGATCA TTGTGCGGGT GGAGATCAAT GGACAGGATC TGAAAATGGA CTGCAAGGAG	840
45	TACAACTATG ACAAGAGCAT TGTGGACAGT GGCACCCACCA ACCTTCGTTT GCCCAAGAAA	900
	GTGTTTGAAG CTGCAGTCAA ATCCATCAAG GCAGCCTCCT CCACGGAGAA GTTCCCTGAT	960
	GGTTTCTGGC TAGGAGAGCA GCTGGTGTGC TGGCAAGCAG GCACCACCCC TTGGAACATT	1020
	TTCCCACTGCA TCTCACTCTA CCTAATGGGT GAGGTTACCA ACCAGTCCTT CCGCATCACC	1080
50	ATCCTTCCGC AGCAATACCT GCGGCCAGTG GAAGATGTGG CCACGTCCA AGACGACTGT	1140
	TACAAGTTG CCATCTCACA GTCATCCACG GGCACGTGTTA TGGGAGCTGT TATCATGGAG	1200
	GGCTTCTACG TTGTCTTGA TCGGGCCCGA AAACGAATTG GCTTTGCTGT CAGCGCTTGC	1260
	CATGTGCACG ATGAGTTCAAG GACGGCAGCG GTGGAAGGCC CTTTTGTCAC CTTGGACATG	1320
55	GAAGACTGTG GCTACAACAT TCCACAGACA GATGAGTCAA CCCTCATGAC CATAGCCTAT	1380
	GTCATGGCTG CCATCTGCGC CCTCTTCATG CTGCCACTCT GCCTCATGGT GTGTCAGTGG	1440

	CGCTGCCTCC GCTGCCTGCG CCAGCAGCAT GATGACTTTG CTGATGACAT CTCCCTGCTG	1500
5	AAGTGAGGAG GCCCCATGGGA GAAAGATAGA GATTCCCCTG GGACCACACC TCCGTGGTTC	1560
	ACTTTGGTCA CAAGTAGGAG ACACAGATGG CACCTGTGGC CAGAGCACCT CAGGACCCCTC	1620
	CCCACCCACC AAATGCCTCT GCCTTGATGG AGAAGGAAAA GGCTGGCAAG GTGGGTTCCA	1680
	GGGACTGTAC CTGTAGGAAA CAGAAAAGAG AAGAAAAGAG CACTCTGCTG CGGGAAATAC	1740
10	TCTTGGTCAC CTCAAATTAA AGTCGGAAA TTCTGCTGCT TGAAACATTCA GCCCTGAACC	1800
	TTTGTCCACC ATTCCCTTAA ATTCTCCAAC CCAAAGTATT CTTCTTTCT TAGTTTCAGA	1860
	AGTACTGGCA TCACACGAG GTTACCTTGG CGTGTGTCCC TGTGGTACCC GGGCAGAGAA	1920
	GAGACCAAGC TTGTTCCCT GCTGGCCAAA GTCAGTAGGA GAGGATGCC AGTTGCTAT	1980
15	TTGCTTTAGA GACAGGGACT GTATAAACAA GCCTAACATT GGTGCAAAGA TTGCCTCTTG	2040
	AATTAAAAAA AAAAAGTACA TTGACTATTT ATACAAATGG GGGCGGCTGG AAAGAGGAGA	2100
	AGGAGAGGGA GTACAAAGAC AGGGAATAGT GGGATCAAAG CTAGGAAAGG CAGAAACACA	2160
	ACCACTCACC AGTCCTAGTT TTAGACCTCA TCTCCAAGAT AGCATCCCAT CTCAGAAGAT	2220
20	GGGTGTTGTT TTCAATGTT TCTTTCTGT GGTTGCAGCC TGACCAAAAG TGAGATGGGA	2280
	AGGGCTTATC TAGCCAAAGA GCTCTTTTT AGCTCTCTTA AATGAAGTGC CCACTAAGGA	2340
	AGTTCCACTT GAACACATGG AATTCTGCC ATATTAATTT CCATTGTCTC TATCTGGAAC	2400
	CACCCCTTAA TCTCTACATA TGATTAGGTC CAGCACTTGA AAATATTCTT AACCNNAATT	2460
25	TGNCTTGGGG GCTTTGCNGN CCAGGTGCTA AAAGGGNTTG GGTAGGNGNC CNCTTNTATN	2520
	TNATNCCTNA AAAGGTTANN G	2541

(2) INFORMATION FOR SEQ ID NO:2:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 501 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Met Ala Gln Ala Leu Pro Trp Leu Leu Trp Met Gly Ala Gly Val	
45	1 5 10 15	
	Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg Ser	
	20 25 30	
	Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp	
50	35 40 45	
	Glu Glu Pro Glu Glu Pro Gly Arg Arg Gly Ser Phe Val Glu Met Val	
	50 55 60	
	Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr	
55	65 70 75 80	
	Val Gly Ser Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser	

	85	90	95
5	Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu His Arg Tyr		
	100	105	110
	Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val		
	115	120	125
10	Tyr Glu Pro Tyr Thr Gln Gly Lys Trp Glu Gly Glu Leu Gly Thr Asp		
	130	135	140
	Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile		
	145	150	155
15	Ala Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp		
	165	170	175
	Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile Ala Arg Pro Asp Asp		
	180	185	190
20	Ser Leu Glu Pro Phe Asp Ser Leu Val Lys Gln Thr His Val Pro		
	195	200	205
	Asn Leu Phe Ser Leu Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln		
	210	215	220
25	Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly Ile		
	225	230	235
	Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg		
	245	250	255
30	Glu Trp Tyr Tyr Glu Val Ile Ile Val Arg Val Glu Ile Asn Gly Gln		
	260	265	270
	Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val		
	275	280	285
35	Asp Ser Gly Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala		
	290	295	300
	Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys Phe Pro Asp		
	305	310	315
40	Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr		
	325	330	335
	Pro Trp Asn Ile Phe Pro Val Ile Ser Leu Tyr Leu Met Gly Glu Val		
	340	345	350
45	Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg		
	355	360	365
	Pro Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala		
	370	375	380
50	Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly Ala Val Ile Met Glu		
	385	390	395
	Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala		
	405	410	415
55	Val Ser Ala Cys His Val His Asp Glu Phe Arg Thr Ala Ala Val Glu		
	420	425	430

Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile Pro
 435 440 445
 5 Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala
 450 455 460
 Ile Cys Ala Leu Phe Met Leu Pro Leu Cys Leu Met Val Cys Gln Trp
 465 470 475 480
 10 Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp
 485 490 495
 Ile Ser Leu Leu Lys
 500
 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2370 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

30	GGCAGCTTG TGGAGATGGT GGACAACCTG AGGGGCAAGT CGGGGCAGGG CTACTACGTG	60
	GAGATGACCG TGGGCAGCCC CCCGCAGACG CTCAACATCC TGGTGGATAC AGGCAGCAGT	120
35	AACCTTCAG TGGGTGCTGC CCCCCACCCC TTCTTGATC GCTACTACCA GAGGCAGCTG	180
	TCCAGCACAT ACCGGGACCT CCGGAAGGGT GTGTATGAGC CCTACACCCA GGGCAAGTGG	240
	GAAGGGGAGC TGGGCACCGA CCTGGTAAGC ATCCCCCATG GCCCCAACGT CACTGTGCCT	300
40	GCCAAACATTG CTGCCATCAC TGAATCAGAC AAGTTCTTCA TCAACGGCTC CAACTGGAA	360
	GGCATCCTGG GGCTGGCTA TGCTGAGATT GCCAGGCCTG ACGACTCCCT GGAGCCCTTC	420
	TTTGACTCTC TGGTAAAGCA GACCCACGTT CCCAACCTCT TCTCCCTGCA GCTTTGTGGT	480
45	GCTGGCTTCC CCCTCAACCA GTCTGAAGTG CTGGCCTCTG TCGGAGGGAG CATGATCATT	540
	GGAGGTATCG ACCACTCGCT GTACACAGGC AGTCTCTGGT ATACACCCAT CCGGGGGGAG	600
	TGGTATTATG AGGTGATCAT TGTGCGGGTG GAGATCAATG GACAGGATCT GAAAATGGAC	660
50	TGCAAGGAGT ACAACTATGA CAAGAGCATT GTGGACAGTG GCACCCACAA CCTTCGTTTG	720
	CCCAAGAAAAG TGGTTGAAGC TGCAGTCATA TCCATCAAGG CAGCCTCTCC ACGGGAGAAG	780
	TTCCCTGATG GTTCTGGCT AGGAGAGCAG CTGGTGTGCT GGCAAGCAGG CACCACCCCT	840
55	TGGAACATT TCCCAGTCAT CTCACCTCTAC CTAATGGGTG AGGTTACCAA CCAGTCCTTC	900
	CGCATCACCA TCCTTCCGCA GCAATACCTG CGGCCAGTGG AAGATGTGGC CACGTCCCAA	960
	GACGACTGTT ACAAGTTTGC CATCTCACAG TCATCCACGG GCACTGTTAT GGGAGCTGTT	1020
	ATCATGGAGG GCTTCTACGT TGTCTTGAT CGGGCCCGAA AACGAATTGG CTTTGCTGTC	1080
	AGCGCTTGCC ATGTGCACGA TGAGTTCAAGG ACGGCAGCGG TGGAGGCCTC TTTTGTCAAC	1140
	TTGGACATGG AAGACTGTGG CTACAAACATT CCACAGACAG ATGAGTCAAC CCTCATGACC	1200

	ATAGCCTATG TCATGGCTGC CATCTGCGCC CTCTTCATGC TGCCACTCTG CCTCATGGTG	1260
5	TGTCAGTGGC GCTGCCTCCG CTGCCTGCGC CAGACAATGG ATGACTTTGC TGATGACATC	1320
	TCCCTGCTGA AGTGAGGAGG CCCATGGGAG AAAGATAGAG ATTCCCCCTGG GACCACACCT	1380
	CCGTGGTTCAC CTTTGGTCAC AAGTAGGAGA CACAGATGGC ACCTGTGGCC AGAGCACCTC	1440
	AGGACCCCTCC CCACCCCACCA AATGCCCTCG CCTTGATGGA GAAGGAAAAG GCTGGCAAGG	1500
10	TGGGTTCCAG GGACTGTACC TGTAGGAAAC AGAAAAGAGA AGAAAAGAGC ACTCTGCTGG	1560
	CGGGAAATACT CTTGGTCACC TCAAATTAA GTCGGGAAAT TCTGCTGCTT GAAACTTCAG	1620
	CCCTGAACCT TTGTCCACCA TTCCCTTAAA TTCTCCAACC CAAAGTATTTC TTCTTTCTT	1680
	AGTTTCAGAA GTACTGGCAT CACACGCAGG TTACCTTGGC GTGTGTCCCT GTGGTACCCG	1740
15	GGCAGAGAAG AGACCAAGCT TGTTCCTCG CTGGCCAAAG TCAGTAGGAG AGGATGCACA	1800
	GTTTGCTATT TGCTTTAGAG ACAGGGACTG TATAAACAAAG CCTAACATTG GTGCAAAGAT	1860
	TGCCTCTTGA ATTAAAAAAA AAAACTAGAT TGACTATTAA TACAAATGGG GGCGGCTGGA	1920
	AAGAGGAGAA GGAGAGGGAG TACAAAGACA GGGAAATAGTG GGATCAAAGC TAGGAAAGGC	1980
20	AGAAAACACAA CCACTCACCA GTCCTAGTT TAGACCTCAT CTCCAAGATA GCATCCCATC	2040
	TCAGAAAGATG GGTGTTGTTT TCAATGTTT CTTTCTGTG GTTGCAGCCT GACCAAAAGT	2100
	GAGATGGGAA GGGTTATCT AGCCAAAGAG CTCTTTTTTA GCTCTCTTAA ATGAAGTGCC	2160
	CACTAAGGAA GTTCCACTTG AACACATGGA ATTCTGCCA TATTAATTTC CAITGTCTCT	2220
25	ATCTGGAACC ACCCTTTAAT CTCTACATAT GATTAGGTCC AGCACTTGAA AATATTCCCTA	2280
	ACCNNAATTN GNCTTGGGG CTTTGCGNGNC CAGGTGCTAA AAGGGNTTGG GTAGGNGNCC	2340
	NCTTNATNT NATNCCTNAA AAGGTTANNG	2370

30 (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 774 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

40 (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

45	Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln	
	1 5 . 10 15	
	Gly Tyr Tyr Val Glu Met Thr Val Gly Ser Pro Pro Gln Thr Leu Asn	
35	20 25 30	
50	Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro	
	35 40 45	
	His Pro Phe Leu His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr	
	50 55 60	
55	Arg Asp Leu Arg Lys Gly Val Tyr Glu Pro Tyr Thr Gln Gly Lys Trp	
	65 70 75 80	

Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn
 85 90 95
 5 Val Thr Val Arg Ala Asn Ile Ala Ala Ile Thr Glu Ser Asp Lys Phe
 100 105 110
 Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala
 115 120 125
 10 Glu Ile Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu
 130 135 140
 Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu Gln Leu Cys Gly
 145 150 155 160
 15 Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly
 165 170 175
 Ser Met Ile Ile Gly Gly Ile Asp His Ser Leu Tyr Thr Gly Ser Leu
 180 185 190
 20 Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
 195 200 205
 Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr
 210 215 220
 25 Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly Thr Thr Asn Leu Arg Leu
 225 230 235 240
 Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser
 245 250 255
 30 Pro Arg Glu Lys Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val
 260 265 270
 Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val Ile Ser
 275 280 285
 35 Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile
 290 295 300
 Leu Pro Gln Gln Tyr Leu Arg Pro Val Glu Asp Val Ala Thr Ser Gln
 305 310 315 320
 40 Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val
 325 330 335
 Met Gly Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala
 340 345 350
 45 Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His Val His Asp Glu
 355 360 365
 Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu
 370 375 380
 50 Asp Cys Gly Tyr Asn Ile Pro Gln Thr Asp Glu Ser Thr Leu Met Thr
 385 390 395 400
 Ile Ala Tyr Val Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu
 405 410 415
 55 Cys Leu Met Val Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Thr

	420	425	430
5	Met Asp Asp Phe Ala Asp Asp Ile Ser Leu Leu Lys Gly Gly Pro Trp		
	435	440	445
	Glu Lys Asp Arg Asp Ser Pro Gly Thr Thr Pro Pro Trp Phe Thr Leu		
	450	455	460
10	Val Thr Ser Arg Arg His Arg Trp His Leu Trp Pro Glu His Leu Arg		
	465	470	475
	Thr Leu Pro Thr His Gln Met Pro Leu Pro Trp Arg Arg Lys Arg Leu		
	485	490	495
15	Ala Arg Trp Val Pro Gly Thr Val Pro Val Gly Asn Arg Lys Glu Lys		
	500	505	510
	Lys Glu Ala Leu Cys Trp Arg Glu Tyr Ser Trp Ser Pro Gln Ile Val		
	515	520	525
20	Gly Lys Phe Cys Cys Leu Lys Leu Gln Pro Thr Phe Val His His Ser		
	530	535	540
	Phe Lys Phe Ser Asn Pro Lys Tyr Ser Ser Phe Leu Ser Phe Arg Ser		
	545	550	555
25	Thr Gly Ile Thr Arg Arg Leu Pro Trp Arg Val Ser Leu Trp Tyr Pro		
	565	570	575
	Gly Arg Glu Glu Thr Lys Leu Val Ser Leu Leu Ala Lys Val Ser Arg		
	580	585	590
30	Arg Gly Cys Thr Val Cys Tyr Leu Leu Arg Gln Gly Leu Tyr Lys Gln		
	595	600	605
	Ala His Trp Cys Lys Asp Cys Leu Leu Asn Lys Lys Lys Leu Asp Leu		
	610	615	620
35	Phe Ile Gln Met Gly Ala Ala Gly Lys Arg Arg Arg Arg Gly Ser Thr		
	625	630	635
	Lys Thr Gly Asn Ser Gly Ile Lys Ala Arg Lys Gly Arg Asn Thr Thr		
	645	650	655
40	Thr His Gln Ser Phe Thr Ser Ser Pro Arg His Pro Ile Ser Glu Asp		
	660	665	670
	Gly Cys Cys Phe Gln Cys Phe Leu Phe Cys Gly Cys Ser Leu Thr Lys		
	675	680	685
45	Ser Glu Met Gly Arg Ala Tyr Leu Ala Lys Glu Leu Phe Phe Ser Ser		
	690	695	700
	Leu Lys Ser Ala His Gly Ser Ser Thr Thr His Gly Ile Ser Ala Ile		
	705	710	715
50	Leu Ile Ser Ile Val Ser Ile Trp Asn His Pro Leu Ile Ser Thr Tyr		
	725	730	735
	Asp Val Gln His Leu Lys Ile Phe Leu Thr Xaa Ile Xaa Leu Gly Gly		
	740	745	750
55	Phe Ala Xaa Gln Val Leu Lys Gly Xaa Gly Xaa Xaa Xaa Xaa Xaa		
	755	760	765

Xaa Leu Lys Arg Leu Xaa

5
770

(2) INFORMATION FOR SEQ ID NO:5:

10 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATGAGTTCA GGACGGCAG

19

25 (2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

40 GGTGCCATAT GTGTCTCC

18

45 Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.
2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the ASP2 polypeptide of SEQ ID NO:2.
3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.
4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.
6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing
5 a ASP2 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.
7. A host cell comprising the expression system of claim 6.
8. A process for producing a ASP2 polypeptide comprising culturing a host of claim 7 under conditions sufficient for
10 the production of said polypeptide and recovering the polypeptide from the culture.
9. A process for producing a cell which produces a ASP2 polypeptide thereof comprising transforming or transfecting
15 a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a ASP2 polypeptide.
10. A ASP2 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence
of SEQ ID NO:2 over its entire length.
11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.
12. An antibody immunospecific for the ASP2 polypeptide of claim 10.
13. A method for the treatment of a subject in need of enhanced activity or expression of ASP2 polypeptide of claim
15 10 comprising:
- (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
20 (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80%
identity to a nucleotide sequence encoding the ASP2 polypeptide of SEQ ID NO:2 over its entire length; or a
nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said
25 polypeptide activity *in vivo*.
- 30 14. A method for the treatment of a subject having need to inhibit activity or expression of ASP2 polypeptide of claim
10 comprising:
- (a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
35 (b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence
encoding said polypeptide; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said
40 polypeptide for its ligand, substrate , or receptor.
- 45 15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of
ASP2 polypeptide of claim 10 in a subject comprising:
- (a) determining the presence or absence of a mutation in the nucleotide sequence encoding said ASP2
polypeptide in the genome of said subject; and/or
50 (b) analyzing for the presence or amount of the ASP2 polypeptide expression in a sample derived from said
subject.
- 55 16. A method for identifying compounds which inhibit (antagonize) or agonize the ASP2 polypeptide of claim 10 which
comprises:
- (a) contacting a candidate compound with cells which express the ASP2 polypeptide (or cell membrane ex-
pressing ASP2 polypeptide) or respond to ASP2 polypeptide; and
(b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the
cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were
not contacted for ASP2 polypeptide activity.
17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.
19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a ASP2 polypeptide.

5

10

15

20

25

30

35

40

45

50

55